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translation are not well understood. Previously, we demonstrated that solvent-accessible volume surrounding a modifiable cysteine increases monotonically with increase in the van der Waal's volume of the adjacent side chain (Lu et al., J.Mol.Biol. 411: 499-510, 2011) and that the magnitude of this effect depends on location within the ribosomal tunnel. using a photocrosslinking approach, we confirm these results. We extend these studies to investigate whether mutations in the nascent peptide deep in the tunnel affect the accessibility of a modifiable reporter cysteine at the exit port and whether specific regions of the tunnel instigate these effects. Tryptophan vis-à-vis alanine, engineered into the nascent peptide at a distance of 17-19 residues from the PTC, alters the accessibility of residues at the exit port, a distance of 33 residues from the PTC, roughly 50 angstroms from the introduced point mutations. These findings are consistent with long-range rearrangements and may contribute to mechanisms governing sequence-specific signaling from different regions of the tunnel during translation. Supported by NIH grant R01GM52302.

1332-Pos Board B224

Coordinated Conformational and Compositional Dynamics Drive Ribosome Translocation

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Translation elongation requires the correlated interplay of both ribosome conformational and compositional dynamics. The compositional factors, elongation factor G (EF-G) and transfer RNA (tRNA), alternately bind to the ribosome to direct protein synthesis, in turn regulating the conformation of the ribosome. The mechanisms for how ribosomal conformation and factor composition are linked, and how these processes dynamically control translocation, remain unclear. Here, we use single-molecule Förster Resonance Energy Transfer (smFRET) with zero-mode waveguides (ZMWs) to correlate directly ribosome conformations and compositions during multiple rounds of elongation at high factor concentrations. Our results show that EF-G•GTP continuously samples both the non-rotated and rotated sates of the ribosome, binding with higher affinity to the rotated state. Upon successful accommodation into the rotated ribosome, the EF-G•ribosome complex evolves through several partially rate-limiting conformational changes and the hydrolysis of EF-G-bound GTP, which results in a ribosome intersubunit conformational change back to the non-rotated state, in turn driving translocation and facilitating both EF-G•GDP and E-site tRNA release from the ribosome. Ribosomal intersubunit conformation discriminates between tRNA•EF-Tu•GTP and EF-G•GTP, with transitions between these two conformations of the ribosome in turn mediated by the factors themselves. These experiments highlight the power of single-molecule methods to track translation, correlating both conformation and composition, at codon resolution in real-time.

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Single Molecule Measurement of Peptide Elongation Rate during Synthesis of a Full-Length Protein

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Ribosomal synthesis of proteins proceeds with pauses that regulate the rhythm of protein synthesis. In order to study the factors that control translation rates, we use the expression of fast maturing Emerald Green Fluorescent Protein (EmGFP) by a reconsitituted *E. coli* cell-free translation system. In order to quantify translation rate in identfied short segments of the sequence, the existing ribosomes and Phe-tRNA^{Phe} in the cell-free mixture are replaced by fluorescent labeled Phe-tRNA^{Phe}(Cy5.5) and L11(Cy3)-ribosomes. Single-molecule FRET trajectories report of multiple accommodations of Phe-tRNA^{Phe}s on single ribosomes during synthesis of EmGFP. An algorithm was developed to identify FRET pulses objectively by anti-correlation of donor and acceptor intensities. The time intervals between two consecutive Phe-tRNA^{Phe}(Cy5.5) FRET pulses can be assigned to particular sequence segments according to their timing relative to two characteristic Phe-Phe doublets near the middle of the EmGFP sequence. Translation proceeds with variable rates which are correlated to codon and isoacceptor tRNA usage. Codon CGG, coding for a rare tRNA^{Arg}, slows elongation approximately 5-fold compared with CGC, coding for a more plentiful tRNA^{Arg}. This difference is eliminated when the total concentration of tRNA^{Arg} isoacceptors is increased. These results quantify the regulation of elongation by tRNA availability. Decreased translational rate due to tRNA selection is concomitant with the emergence of an upstream nascent polypeptide from the ribosomal exit tunnel. Thus, the rhythm of translation can have an upstream impact on co-translational processes such as protein folding.
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DNA and RNA Structure I

1334-Pos Board B226

Energy-Tunable Quantitative Hybridization Assay

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We describe here a novel molecular design approach to optimizing the sensitivity and selectivity of probe-target interactions. The methodology employs a novel hybridization assay which uses a unique class of energy tunable competitor strands (C*) that hybridize to a probe strand (P). The assay is based on competitive binding equilibria for a common probe strand (P) between such competitor strands (C*) and a target strand (T). We demonstrate that families of tunable C*P complexes exhibit enhanced discrimination between targets and mismatched targets, thereby reducing false positives/negatives. The methodology also allows quantification of target strand concentrations, a determination heretofore not readily available by conventional hybridization assays. We present solution data that establish proof-of-principle for this energy-tunable quantitative hybridization assay. It is envisioned that future practical applications of this technology will be based on surface bound/spatially resolved DNA arrays.

1335-Pos Board B227

Capillary Electrophoresis as a Probe of Counterion Condensation Theory

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Electrophoresis is a useful probe of DNA electrostatics, because the mobility is related to the ratio of the effective charge of the DNA to its frictional coefficient. Manning has developed a theory describing the electrophoretic mobility of DNA as a function of various experimental parameters, based on counterion condensation theory. The mobility is predicted to vary linearly with cation valence, the logarithm of ionic strength, and the logarithm of the axial charge spacing along the contour length. Since the theory contains no adjustable parameters, the dependence of DNA mobility on various experimental variables can be used to probe the fundamental correctness of the underlying theory. For double-stranded DNA, the observed mobilities vary linearly with cation valence and the logarithm of ionic strength, as predicted by the Manning theory. The calculated and observed mobilities agree within ~5% if b is equal to 1.7, the axial charge spacing of dsDNA. For single-stranded DNA, the observed mobilities vary linearly with the logarithm of ionic strength and the logarithm of the fractional charge density of the backbone, as predicted by the Manning theory. However, the calculated and observed mobilities do not agree unless the value of b decreases with increasing ionic strength, raising the question of the physical meaning of this parameter.

1336-Pos Board B228

Characterization of Aggregates Formed from Oligonucleotides in the Presence of a Surfactant

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Characterization of aggregates formed from oligonucleotides of 20 to 100 bases in length in the presence of the surfactant, CTAB (cetyl trimethylammonium bromide) has been performed. UV spectroscopy was used to observe changes in the absorbance spectra of single and double stranded DNA in the presence of CTAB as a function of temperature and CTAB concentration. The results indicate that the spectral changes are a result of light scattering from various size aggregates formed between DNA and CTAB when the ratio of CTAB to DNA concentration is about 0.5 or larger. Electrophoresis has been used to compare the mobilities of both double and single stranded DNA in the presence of CTAB and the results show that the DNA - CTAB samples give rise to broad bands in the gel images indicative of a range of aggregate sizes. Atomic force microscopy was used to obtain a topographical view of the aggregates that were dried on single crystal silicon surfaces and reconstituted in water. The aggregates in solution were apparent in optical images and both optical and atomic force microscope images showed that the aggregates were non-spherical and that they varied in size from nanometers to microns.

1337-Pos Board B229

Duplex RNA and DNA/RNA Hybrid Condensation by Multivalent Ions

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Due to the biological significance of DNA condensation and the potential use of packaged RNA for therapeutics, the behavior of nucleic acids in the presence of condensing agents is a topic of great interest. Following the discovery that double-stranded RNA resists condensation in solutions containing cobalt